Electrical Penetration Graph from *Cicadulina mbila* and its application in plant breeding to screen a potential resistance of maize to MSV transmission.

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Abstract:

A thorough knowledge of *C. mbila* feeding behavior is necessary to understand MSV epidemiology and to breed efficiently for plant resistance to the insect. This will contribute to the improvement of the resistance to maize streak disease. The MSV transmission efficiency of *C. mbila* decreased on some maize inbred lines from IRAT 297. The feeding activity of *C. mbila* on maize has been electronically monitored using the DC system combined with simultaneous videofilming. Signal characterization was carried out with temporal, spectral, and time frequency analysis. The complete stylet pathway was obtained by optical and electron microscopy observations of serial sections of the leaf tissue containing the inserted stylets. Based on these techniques seven patterns of waveform were identified and grouped into five classes. Each class was correlated with plant tissue localization and presumed insect activity. There were enough discriminant quantitative variables in spectral and temporal analysis of digital data to proceed to an automatic classification of EPG graphs. This software will help to compare resistant and susceptible maize inbred lines by testing a large number of plants. The preliminary results of this comparison and its epidemiological implications are discussed.

Introduction:

Cicadulina mbila is a major pest of maize in Africa, due to its ability to transmit efficiently maize streak virus (MSV). A thorough knowledge of *C. mbila* feeding behavior is necessary to breed efficiently for plant resistance to the insect, in order to

improve the resistance to maize streak disease. Only recently the feeding activity of *C. mbila* had been electronically monitored using the AC system (MESFIN *et al.* , 1995) or the DC system (KIMMINS et BOSQUE-PEREZ, 1996). However the different waveform patterns were not correlated to tissue localisation . Some resistance , which affect settling, probing and oviposition behaviour of *C. mbila*, were founded in African and Mascarenes genotypes (KAIRO *et al.*, 1995). We have previously demonstrated that resistant material against *C. mbila* exist in Reunion ecotypes (REYNAUD, 1988) . Resistance against *P. maidis* affect MMV transmission (BUDUCA, 1995). It would be interesting to know if the resistance against *C. mbila* is efficient enough to affect MSV transmission at an accurate epidemiological level.

This work has 5 targets:

- determination of the effect of the insect resistance on the transmission efficiency of MSV
- classification of the different EPG waveforms correlated with cell localisation of the stylets and insect activity
- choice of the reproductive spectral and temporal parameters to characterize the EPG waveform
- automation of EPG waveform recognition in order to use it for breeding
- comparison of the feeding behavior of viruliferous *C. mbila* on resistant and susceptible inbred lines

Materials and methods:

Insects:

For all the experiments, young adults of an active selected biotype of *C. mbila* from Reunion Island were used. For EPG studies only the females were wired.

Virus isolate:

The virulent isolate N2A from Saint-Pierre (La Réunion)(PETERSCHMITT *et al.*, in this volume) was used for all the studies except for the experiment concerning different durations of IAP (isolate B422-1,(Lefrère, 1993))

Plant genotypes :

Sabrina (France- Mais) is a temperate maize hybrid use as susceptible check. A211, D212, D255, 37-2, 37-3, 36-1 and 36-2 are inbred lines extracted from IRAT 297 (HAINZELIN et MARCHAND, 1986) . MP705 is a american inbred line (reg. n° GP 130, USDA-ARS).

Transmission study in insect-proof screenhouses :

The experimental design consisted of two complete randomized replications. Each plot had 36 plants. During IAP, plants were covered by individual small cages. At the end of the IAP, a insecticide was sprayed on the plants (Piperonylbuytoxyde and pyrethrins).

EPG procedure:

The feeding activity of *C. mbila* on maize has been electronically monitored using the DC system (Giga 4 amplifier, Wageningen Agri. Univ.) . Young females were monitored for 2 hours minimun and signals were converted into digital data. The adults were simultaneously videofilmed to visualized honeydew excretion. Signal characterization was carried out after converting it into digital data (Viewdac, Kheitley) with temporal, spectral, statistic and time frequency analysis (TFview, Matlab). The stylets were amputated by microcautery (CA-50, Syntech) during recording of each signal class. The complete stylet pathway was obtained by optical and electron microscopy (Phillips)observations of serial sections of the leaf tissue containing the inserted stylets.To obtained the complete stylet pathway, serial optical observations of semi thin cross sections were made. When the first damaging cell or the salivary sheath has been reach, serial electron microscopy observations of ultra-thin cross sections were made until the observation of the feeding point.

Results and Discussion :

Transmission study in screenhouse :

In the two experiments the percentage of infected plant were significantly ($p < 0.0001$) different between the genotypes and between the inoculation conditions. The percentage of infected plant increased significantly with the increasing of the number of insects or the duration of IAP both for the susceptible check (Sabrina) and

the inbred lines (A211, D212, D255). Nevertheless, the resistant inbred lines required a higher number of insects ora longer duration of IAP before the curves of infected plant reached a plateau. In our experimental conditions, 20 hours of presence of *C. mbila* on the plant were necessary to obtain the maximum transmission rate of MSV on a susceptible hybrid. So a resistance to MSV transmission could be efficient in natural conditions particularely if maize is only an alternative host plant of *C. mbila* .

Classification of EPG waveform:

Seven patterns of waveform were identified and grouped into five classes (Table 1). Class 1 signal appeared as soon as the stylets penetrate a leaf. It was a complex signal as shown by the large spectral area between 0 and 10 Hz. The main frequency was around 1 Hz and was similar to salivary pump frequency of *Acyrthosiphon pisum* (TJALLINGII,1995). During this signal the stylets were generally localized in the mesophyll but also in vascular tissue when it occured between class 2 and class 3 signals. Our observations suggested that class 1 signal corresponded to stylet activities and sheath salivation .The direct intracellular stylet track explained the short duration of class 1 signal.

The class 2 signal was always preceded by a class 1 signal. It was a periodic signal , whose fundamental was around 6 hz, very similar to class 2 signal from *P. maidis* (BUDUCA *et al*., 1996) and to pattern G from aphids (TJALLINGII, 1995). During class 2 signals, the stylets were observed in mesophyll cells or in xylem. Class 2 signal always started by a very short potential drop which suggested the piercing of a new cell or vessel. When *C. mbila* was feeding in a mesophyll cell we observed damaged plasmic membrane, an empty cytoplasm and some desorganised chloroplasts adjacent to the feeding hole suggesting an active ingestion. For aphids pattern G was correlated with active ingestion in xylem only and it was generated by the electromotive forces of muscles activating the pharyngeal pump. The similar frequencies of the signals recorded with sternorryncha and auchenorryncha during their active feeding in the same tissues suggested that class 2 signal correspond also to the pharyngeal muscular activities of *C. mbila*. *C. mbila* produced either numerous class 2 signals of short duration (2 or 3 mn) between class 1 signals or a more longer class 2 signal (up to 30 mn) with excretion of honeydew and often

followed by a class 3 signal. The durations of signals and their sequence could indicate the localisation of feeding: short class 2 signals suggested mesophyll active ingestion whereas a longer class 2 signal suggested a xylem active ingestion.
Based on stylectomy and electron microscope observation, the class 3 and class 4 signals were related to phloem localization. However with class 4 signal there was much more salivary material in the phloem cells when compared to class 3. Futhermore, some callose deposits were observed in some sieve pores with class 4 signal. The class 3 signal was very regular with low amplitude and only low frequencies with a peak around 0.7 Hz. Duration of class 3 signal was very constant and around 30 mn. Class 4 signal always followed class 3 signal and could be recorded without interruption for several hours. Class 4 signal was a periodic signal with a fundamental between 5 and 7 Hz, similar to class 2 signal but its relative amplitude was ten times lower. Sometimes the waveform differ slightly and spectral analysis showed an additionnal peak around 0.7 Hz of variable relative amplitude. During class 4 signal *C. mbila* produced basic honeydew and remained still in a position where it was very close to the leaf epidermis. PRADO and TJALLINGII (1994) defined also two signal classes for aphids with phloem localization : E1 correspont to aqueous salivation and E2 correspont to passive ingestion with some time of salivation. Class 3 and class 4 signals were similar to respectively with E1 and E2 with respect to TEM observations and insect activities deduced from similar signal frequencies. *C. mbila* was capable of feeding in mesophyll cells. However according to the large proportion of time during which class 4 signal was recorded on a maize genotype that we believe to be susceptible to the insect, it prefers phloem. Class 5 signal was characterized in temporal mode by a very low amplitude signal interupted about every 20 s by a large peak. It was rarely recorded and only intercalated in class 1 or class 2 signals. KIMMINS (1989) founded a similar pattern (P6) produced by *Nilaparvata lugens* especially on resistant rice genotypes. Thus, the analysis of the insect activities and stylets localization during class 5 signal could be important for studying varietal resistance .

Programmation of a software to automate EPG waveform recognition :

Comparison of the feeding behavior of *C. mbila* on resistant and susceptible inbred

lines:

The preliminary results showed that *C. mbila* had more non-probing activities on some inbred lines (A211, 37-3, MP 705) . The insects produced also more class 1 and class 5 signal on this inbred lines. On inbred ligne A211, *C. mbila* made more stylet penetration and produced class 3 signal more later. Salivation in phloem vessels occurred during class 3 signal. As showned by KIMMINS *et* BOSQUE-PEREZ (1996) this signal appearence is necessary to transmit MSV. This first results could be related to previous observations in screenhouse where we demonstrated that *C. mbila* needed a longer period to transmit MSV to resistant inbred lines.

Conclusions:

A first classification of *C. mbila* EPG waveforms correlated with plant tissue localisation of the stylets, had been etablish. Interestingly it is very similar to aphid classification. The spectral and temporal variables are discriminant enough to proceed to a automation of EPG waveform recognition. A software will be soon achieved in order to use it in breeding schemes for insect resistance. The feeding behavior of *C. mbila* on some inbred lines is different enough to discriminate the resistance to virus transmission with this technique . Futher comparisons of resistant material will be carry on by EPG tests to confirm this preliminary results. It will be also use to check the selection of this resistance to transmission character in homozygous lines. The efficiency of this system of resistance, in order to use it in breeding program, must be previously studied in different epidemiological conditions.

References:

BUDUCA C., 1995. Etude du comportement alimentaire de Peregrinus maidis (Ashmead, 1890) par electropénétrographie, en relation avec la resistance à la mosaïque du maïs . Thèse de docteur en sciences , USTL, Montpellier, 93 p.

BUDUCA C., REYNAUD B., LAN SUN LUK D., MOLINARO F., 1996 - Electrical Penetration

Graphs from *Peregrinus maidis* (Ashmead) (Homoptera : Delphacidae) on a susceptible maize hybrid. Entomol. Exp. Appl., 79, 131-139

HAINZELIN, E.M.,MARCHAND J.L., 1986. Registration of IRAT 297 Maize Germplasm. Crop Science. 26**:** 1090-1091.

KAIRO M.T.K., KIDUYU P.K., MUTINDA C.J.M. & EMPIG L.T., 1995. Maize streak virus: evidence for resistance against Cicadulina mbila Naude, the main vector species; Euphytica, 89,109-114.

KIMMINS, F.M., 1989. Electrical Penetration Graphs from Nilaparvata lugens on Resistant and Susceptible Rice Varieties. Entomol. Exp. Appl. 50**,** 69-79.

KIMMINS F.M., BOSQUE-PEREZ N.A., 1996. Electrical penetration graphs from Cicadulina spp. And the inoculation of a persistent virus into maize. Ent. Exp. Appl. , 46-49.

LEFRERE P., 1993. Importance des conditions d'inoculation du maize streak virus par Cicadulina mbila pour l'évaluation de la resistance du maïs à la striure. Mémoire de DEA, ENSAIA Nancy, 47 p.

MESFIN, T.,DEN HOLLANDER J. & MARKHAM P.G., 1995. Feeding activities of Cicadulina mbila (Hemiptera: Cicadellidae) on different host-plants. Bull. Entomol. Res., 85, 387-396.

PRADO, E.,TJALLINGII W.F., 1994 Aphid activities during sieve element punctures . Entomol. Exp. Appl. 72**,** 157-165.

REYNAUD B., 1988. Transmission des virus de la striure, du stripe et de la mosaïque du maïs par leurs vecteurs *Cicadulina mbila* (Naude, 1924) et *Peregrinus maidis* (Ashmead, 1890) (Homoptera). Thèse de docteur en sciences, USTL, Montpellier, 173 p.

TJALLINGII, W.F., 1995.Regulation of phloem sap feeding by aphids. In R.F. Chapman Gerrit de Boer (ed.) Regulatory mechanism in insect feeding. Chapman & Hall , New York.

Table 1: Characterization of EPG waveform on susceptible maize hybrid cv Sabrina

EPG waveform	Amplitude (volt)	Frequencies (Hz)	Mean duration	Plant tissue	Presumed insect activity	Honeydew excretion
classe 1 (A)[(1)]	> 5	not relevant	5 s	epidermis	stylet contact	no
classe 1 (B)	1,5	0,5 [(2)]	5 mn	all tissue	sheath salivation	no

classe 1 (C)	1,5	0 - 10 [3]	5 mn	all tissue	stylet activities	no
classe 2 (G)	3	5-7 [2]	1 -30 mn	mesophyll or xylem	active ingestion	1 drop every 10 mn
classe 3 (E1)	0,7	0,5 - 1[2]	30 mn	phloem	watery salivation	no
classe 4 (E2)	0,4	0,5 - 1[2] and 5-7[2]	+ 1 h	phloem	watery salivation and passive ingestion	1 drop every 5 mn
classe 5	1,5	0.05[2]	1 - 10 mn	not determined	stylet activities and rest	no

(1): aphids classification

(2) : peak

(3) : spectral area